

# PATENT SPECIFICATION

NO DRAWINGS

Inventor: BANOO ZERXES LASHKARI

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## COMPLETE SPECIFICATION

### Process for the Preparation of Cheese Flavour Compositions

We, UNILEVER LIMITED, a Company registered under the laws of Great Britain, of Port Sunlight, in the County of Chester, England, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to the preparation of a flavouring substance, particularly to the preparation of a cheese flavour composition.

The present invention provides a process for the preparation of a composition having the flavour of cheese, in which micro-organisms capable of producing a cheese flavour are grown in a high-fat medium.

The micro-organism employed may be any non-pathogenic lipolytic micro-organism that is capable of developing a cheese-like flavour. In particular *Penicillium* sp. (especially *Penicillium roqueforti*), *Candida lipolytica*, *Oidium lactis*, *Cladosporium* sp. (especially *Cladosporium butyri*), *Micrococcus* sp. and *Lipomyces* sp. give good flavours.

The high-fat medium selected for production of the cheese flavour suitably contains between 30% and 80% fat, preferably between 40% and 60% fat.

The fat may be of animal origin, for example, that separated from milk, or of vegetable origin, for example, coconut oil or soya-bean oil. The preferred fat for use according to the invention, is that obtained from the milk of the cow or buffalo.

The high-fat medium should contain, in addition to the fat, sufficient carbohydrate, protein, mineral salts, trace elements, vitamins and other growth factors as may be necessary to sustain the growth of the micro-organism employed. A particularly suitable high-fat medium which in general fulfills these re-

quirements as to content of fat and other nutrients is cow or buffalo cream.

The high-fat medium may be inoculated by transferring to it young actively growing cells of the chosen micro-organism, preferably after having been sub-cultured in a suitable liquid or solid sub-culture medium.

Liquid sub-culture medium containing actively growing cells may be added either directly to the high-fat medium, or the cells may be separated from their supernatant, optionally washed and then added to the high-fat medium. In the latter case the cells may be separated, for example, by centrifugation.

When a solid sub-culture medium is employed the cells may conveniently be cultured on the surface of the medium, scraped off when in an actively growing state, optionally washed and then transferred to the high-fat medium.

A particularly suitable sub-culture medium for preparing young actively growing cells, adapted for producing later an intense cheese flavour in the high-fat medium, is one having a low protein content. Desirably, the sub-culture medium should contain from 0.1% to 3.0% protein, preferably from 1.0% to 2.0%. We have found that undesirable putrid notes may develop with sub-culture media containing more than 3% protein.

The sub-culture medium may be adjusted to any pH at which the micro-organisms will grow, but preferably the pH is adjusted to a value within the range pH 4.0 to 5.0. A particularly suitable sub-culture medium is whey broth having a pH of 4.5.

The sub-culture medium may be incubated at a temperature optimum for growth of the selected micro-organism. In general this is between 20° and 40°C. It is usually necessary to incubate this medium for a period not

exceeding 7 days, a particularly suitable time being about 48 hours.

When a liquid sub-culture medium is used it may be incubated without stirring, but we have found that it is preferable to agitate and aerate in order to obtain an actively growing culture suitable for producing an intense cheese flavour after transfer to the high-fat medium.

When a solid sub-culture medium is used, aerobic incubation is preferably employed.

As an alternative, the use of a sub-culture medium may be omitted and the high-fat medium may be inoculated with cells or spores of the selected organism which are not necessarily in an active state of growth. Thus, resting or dormant cells or spores may be transferred directly to the high-fat medium and incubated under conditions suitable for producing the desired cheese flavour, without recourse to a sub-culture medium. However, the quality of the cheese flavour obtained by employing such a process, although adequate for most purposes, may not be so good as that obtained when employing a sub-culture medium to condition a culture of actively growing cells for transfer to the high-fat medium.

The inoculated high-fat medium is suitably incubated at a temperature between 25°C and 40°C, preferably at about 28°C to 32°C. Although incubation may be carried out without stirring, it is generally preferred to apply agitation with aeration in order to develop a good cheese flavour free from off-odours. The incubation should be continued until a highly cheesy aroma is obtained. This is usually after about 48 hours, but it may be necessary to vary the time of incubation depending on the temperature, rate of agitation and aeration and on the vigour of the selected strains of micro-organisms.

The highly flavoured composition so obtained may be sterilised, for example by heating, and then added directly to food without any further treatment. If not required for immediate use it may be dried, for example by spray drying.

The following Examples are included to illustrate the invention.

#### EXAMPLE I

##### Preparation of Inoculum

Whey broth medium (pH 4.5) was inoculated with a vigorously growing culture of *Candida lipolytica* previously grown for 48 hours on tomato tryptone agar. Approximately 30,000,000 cells from this culture were added to every 50 ml. of whey broth medium. This broth was incubated at 24°C to 26°C under conditions of constant agitation and aeration. For the latter purpose, sterile air was introduced into the fermentation vessel at the rate of 1.2 litres of air per minute. In

order to control foaming, 0.05% of silicone oil was added.

After 24 hours of incubation, sufficient amount of fresh, sterile whey broth was added to make up for loss due to evaporation. The fermentation was allowed to proceed for 24 hours more, making a total fermentation time of 48 hours.

#### Preparation of Flavouring Compositions

Approximately  $2.15 \times 10^{10}$  of cells of the broth grown *Candida lipolytica* were used for inoculating 200 gm. of sterile buffalo cream containing 50% fat. The inoculated cream was shaken and aerated at 37°C. After 48 hours, the inoculated cream had acquired a highly cheesy aroma and was used as the basic material for preparing cheesy ghee.

#### EXAMPLE 2

The procedure of Example I was repeated except that the micro-organism employed was *Oidium lactis*, and temperature of incubation of both the whey broth and cream cultures was between 24°C and 40°C.

#### EXAMPLE 3

The procedure of Example I was repeated except that the micro-organism employed was *Cladosporium butyri* and the whey broth culture was incubated at about 25°C for 72 hours. A strong cheesy flavour was obtained.

#### EXAMPLE 4

The procedure of Example I was repeated except that the micro-organism employed was *Micrococcus* sp. which was used to inoculate the whey broth medium. This culture was incubated for 4 days at a temperature of about 30°C to 37°C.

#### EXAMPLE 5

This Example illustrates the use of *Penicillium roqueforti* in conjunction with *Candida lipolytica* for the production of a blue-cheese type flavour.

Cells of *Candida lipolytica* from agar slants were inoculated directly into sterile cream and incubated at 30°C for 48 hours with the usual aeration. After this time, the cream was heated at 80°C for 30 minutes. It was then cooled to 40°C and spores of *Penicillium roqueforti* (approximately 30,000,000 per 100 gm. of cream) were added. The cream was further incubated for 24 hours. At the end of this time a strong blue-cheese flavour had developed and the cream was spray-dried and stored.

#### WHAT WE CLAIM IS:—

1. A process for the preparation of a cheese flavour composition, in which micro-organisms capable of producing a cheese-flavour are grown in a high-fat medium.

2. A process according to Claim 1, in

- which the high-fat medium contains from 30% to 80% fat.
3. A process according to Claim 2, in which the high-fat medium contains from 40% to 60% fat.
- 5 4. A process according to any of Claims 1 to 3, in which the fat is animal fat.
5. A process according to Claim 4, in which the fat is milk fat.
- 10 6. A process according to any of Claims 1 to 3 in which the fat is vegetable fat.
7. A process according to any of Claims 1 to 5, in which the high-fat medium is cream from cows' milk.
- 15 8. A process according to any of Claims 1 to 5, in which the high-fat medium is cream from buffalo milk.
9. A process according to any of Claims 1 to 8, in which the micro-organism is grown in a sub-culture medium immediately before transfer to the high-fat medium.
- 20 10. A process according to Claim 9, in which the sub-culture medium contains from 0.1% to 3.0% protein.
- 25 11. A process according to Claim 10, in which the sub-culture medium contains from 1.0% to 2.0% protein.
12. A process according to any of Claims 9 to 11, in which the sub-culture medium has a pH of between 4 and 5.
- 30 13. A process according to Claim 12, in which the sub-culture medium is whey broth having a pH of about 4.5.
14. A process according to any of Claims 1 to 13, in which the micro-organism is *Penicillium* sp.
15. A process according to any of Claims 1 to 13, in which the micro-organism is *Candida lipolytica*.
16. A process according to any of Claims 1 to 13, in which the micro-organism is *Oidium lactis*.
17. A process according to any of Claims 1 to 13, in which the micro-organism is *Cladosporium* sp.
18. A process according to any of Claims 1 to 13, in which the micro-organism is *Micracoccus* sp.
19. A process according to any of Claims 1 to 13, in which the micro-organism is *Lipomyces* sp.
20. A process according to any of Claims 1 to 19, in which the cheese flavour composition is dried by spray drying.
21. A cheese flavour composition obtained by the process claimed in any of the preceding claims.
22. A cheese flavour composition obtained by the process claimed in any of Claims 1 to 20 and substantially as hereinbefore described in any of the Examples.
23. A method of flavouring a foodstuff in which there is incorporated into the foodstuff a small proportion of the cheese flavour prepared according to the process claimed in any of Claims 1 to 20.
24. A flavoured food product obtained by the method of Claim 23.
- UNILEVER LIMITED  
R. JONAS,  
Agent for the Applicants.

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